



## Extraction of ethanol with higher alcohol solvents and their toxicity to yeast

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### ABSTRACT

In a solvent extraction screening study, several  $\beta$ -branched alcohols in the 14–20 carbons range show improved extractive performance to recover ethanol from aqueous solutions compared to commonly studied solvents such as oleyl alcohol and 1-dodecanol. These  $\beta$ -branched alcohols were selected for screening based on extrapolation of results in earlier work with lower molecular weight aliphatic alcohol solvents, that indicated higher separation factors should be realized when hydroxyl position is mid-chain, and there is branching. Solvent toxicity to a commercial yeast commonly used in fuel ethanol production also was evaluated for these as well as several lower molecular weight alcohols. For the alcohols studied, those containing 12 or fewer carbons were toxic or inhibitory to the yeast; those containing 14 or more carbons were non-toxic and non-inhibitory.

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### 1. Introduction

The use of renewable feedstocks for conversion to transportation fuels is growing rapidly. For fuel ethanol, following high-starch grains, the next large source of feedstock is lignocellulosic biomass [1]. A difficulty with this source is that the fermentable C6 sugars that result from hydrolysis are typically less concentrated than those that are derived from high starch grains. Significant amounts of soluble hemicellulose drive up the viscosity in the fermentor. In addition, fermentation inhibitors may be present, though this depends on the pretreatment hydrolysis method [2–4]. For these reasons, lignocellulose-based fermentations are more dilute than high starch grain-based fermentations, and the alcohol product is necessarily significantly less concentrated [5,6]. This is a problem when distillation is used as the alcohol recovery method. As distillation feed concentration drops (*i.e.* for lignocellulosic feedstocks), distillation energy use (and cost) rises exponentially [7,8]. In a typical fuel ethanol plant in the U.S., the fermentation broth is distilled in a system using a beer column and a rectification column, then dehydrated by pressure-swing adsorption using molecular sieve adsorbents. A well-integrated process requires approximately 15,600 BTU/US gallon (4350 MJ/m<sup>3</sup>) of anhydrous ethanol [8–10]. This is equivalent to ~20% of the energy content of the ethanol produced, based on the lower heating value (LHV) of 75,700 BTU/US gal

(21,100 MJ/m<sup>3</sup>). The LHV assumes that the latent heat of vaporization of water in the fuel and the reaction products is not recovered. It is useful in comparing fuels where condensation of the combustion products is impractical, as in automobile engines.

There are a variety of alternatives to distillation for recovering ethanol from aqueous solutions such as fermentation broths [11,12]. These include membrane permeation, vacuum stripping, gas stripping, solvent extraction, adsorption and various hybrid processes. Depending on the concentration of the feed solution and other factors, some of these methods have the potential to be less energy intensive than distillation. For instance, Othmer and Ratcliffe [13] showed substantial energy savings for solvent extraction over distillation for recovering ethanol from dilute feeds of 2% ethanol, and lesser savings for 5% feeds. A reduction in this separation energy requirement would increase the net energy ratio for ethanol production, reduce carbon emissions, and reduce operating costs.

This paper describes a search for high performance solvents to be used in liquid–liquid solvent extraction of ethanol from dilute aqueous solutions. There are many important criteria for selecting a liquid–liquid extraction solvent, and these criteria can differ depending on the extraction methodology. For instance, in an extractive fermentation process, the solvent contacts the fermentation broth or a filtered portion of the broth, and after disengagement from the solvent, the aqueous phase is returned to the fermentor. In this case, some important criteria are [14]: (1) good extraction performance (*i.e.*, partitioning of the product between the solvent and aqueous phases), (2) low solvent solubility in the aqueous phase, (3) low solvent toxicity (to workers, to the environment, to fer-

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mentation microorganisms), (4) effective product recovery from the solvent and its regeneration, (5) rapid phase separation, (6) chemical stability, (7) acceptable solvent handling properties (low melting point, compatible with preferred materials of construction), and (8) low formation of stable emulsions or foams. Also, the solvent should not be a viable carbon source for microorganisms (contaminants, or the primary organism itself) that may be present in the fermentation system, as this will contribute to solvent loss. Instead of direct mixing of the aqueous and solvent phases, a membrane contactor may be used, thereby eliminating the need for solvent–aqueous phase disengagement, and the need to balance the flows of the two phases to avoid flooding conditions in the extractor. This can remove the need for the criteria of rapid phase-separation and minimization of stable emulsions or foams. Another case is extraction from an aqueous phase that will not be returned to the fermentor, which may reduce or eliminate the requirement that the solvent be non-toxic to the fermenting microorganism. It is common for ethanol plants using grain feedstocks to produce a high-protein animal feed as a co-product. For this application, any residual solvent in this co-product must be safe for the animals to which it is fed, and not diminish the value of products derived from those animals.

Our strategy for solvent selection starts with extraction performance at a particular operating condition (temperature and aqueous phase concentration) to quickly generate a comparison for many solvents, and a short list of solvents for further study. While it might be more realistic to carry out screening tests using fermentation media, the use of ethanol/water systems gives a good relative ranking of solvent performance. Additionally, most of the data reported in the literature is for ethanol/water systems, which is useful for comparison purposes. For the case of an extractive fermentation process, an evaluation of the toxicity or inhibitory behavior of a set of solvents to the fermenting microorganisms then is carried out on high priority solvents. Additional studies to investigate a few solvents in depth against the rest of the above criteria, and using fermentation broth then become more manageable. It is known that the salts present in the fermentation media will modify ethanol distribution coefficients, as could various organic fermentation components and yeast cells. Ultimately, detailed phase diagram information will be needed to define extraction performance over the full range of expected operating conditions.

Several researchers have ranked solvent classes based on experimental comparisons of performance: carboxylic acids > alcohols > esters > amines > ketones > ethers > hydrocarbons [15–17]. Alcohols are one of the better classes of solvents. However, prediction of the relative performance of different alcohols has had mixed results. It has been shown that the ethanol capacity of the alcohol decreases as molecular weight increases. Structural differences such as branch position and size have an effect [15,16]. In addition, we have shown that position of the hydroxyl group [18,19] has a significant effect on performance due to the extended hydrogen-bond structure of the solvent molecules. Using this information, for alcohol isomers of the same molecular weight, improved separation factor would be expected for the more highly branched secondary or tertiary alcohols with long primary and branch chains.

In the present work,  $\beta$ -branched alcohols are compared with several commonly studied alcohols for solvent extraction performance. The  $\beta$ -branched alcohols are made by the Guerbet reaction or the oxo process. The Guerbet reaction converts a primary aliphatic alcohol into its  $\beta$ -alkylated dimer alcohol. Most commonly used are alcohols of natural origin, which contain an even number of carbons. Oxo alcohols are derived from the hydroformylation of linear olefins. An attractive feature of the C14–C20 alcohols

is that they would be expected to have very low solubility in the aqueous phase and low toxicity to fermenting microorganisms. Their branching causes them to be liquids at room temperature; whereas, the unbranched *n*-alcohols of comparable molecular weight are solids. In addition, because these solvents have high boiling points of >290 °C (>563 K), recovery of the extracted ethanol may be by a simple flash process or a few distillation stages. It should also be noted that three  $\beta$ -branched alcohols were included in a study by Ishii et al. [20] in solvent extraction of *n*-butanol, and high butanol partition coefficients were obtained.

Regarding the toxicity of alcohols to yeast, low molecular weight alcohols (C2–C10) are toxic or inhibitory (to yeast growth and/or ethanol production), while higher molecular weight alcohols are not [21]. There is inconsistency at intermediate molecular weights (*i.e.*, 10–12 carbons). The variation in this range is probably due to differences in testing methods, analysis methods, yeast varieties, and solvent concentrations. It has been shown that preventing yeast exposure to the solvent interface reduces toxicity because yeast is exposed only to the solvent dissolved in the aqueous phase. This has been done by separating the yeast from the solvent with porous membranes [22], or by encapsulating the yeast in alginate beads along with a vegetable oil [23]. In another study, some solvents that were complete inhibitors of yeast growth at saturation in the aqueous phase were partially or not inhibitory at 10% of saturation [24]. Finn [25] showed that for paraffinic hydrocarbons, inhibition of cell growth depends on the concentration of the hydrocarbon in the aqueous phase. While a C11 paraffin was toxic and a C12 was non-toxic, a C11 diluted to the same concentration as the C12 at saturation was non-toxic. Log *P*, the logarithm of the octanol–water partition coefficient for a solvent, has been used to predict toxicity [26]. For the solvents tested, a graph of metabolic activity for *S. cerevisiae* shows a breakpoint at log *P* = 5–6, with low or zero activity below this region and full activity above it. For reference, the log *P* of 1-decanol is 4.6, 1-dodecanol is 5.1, and 1-tetradecanol is 6.0. A good review of solvent cytotoxicity was done by Salter and Kell [27]. This review includes the probable mechanism (disruption of membrane processes due to solubilization of solvent into the cell membrane), and predictors of cytotoxicity. They make the point that log *P* is not a good predictor of solvent toxicity in the range of 2–4 when considering many types of solvents, but is useful for specific solvent classes.

## 2. Experimental

### 2.1. Measurement of ethanol partitioning

The solvent screening technique developed previously [28] was employed to measure the partition of ethanol and water between an aqueous phase, initially 5 wt% ethanol, and the solvent phase. At least two extraction runs were carried out for each compound, and the results averaged. Extractions were at 33 °C with an aqueous-to-organic phase volume ratio of 2:1 and a total liquid volume of 7.5 ml. The mixtures were emulsified multiple times to ensure that equilibrium was reached, then phase separated by centrifugation at the extraction temperature. Gas chromatography using a thermal conductivity detector and an internal standard method was employed to determine the equilibrium ethanol and water concentrations in the organic phase, and the ethanol concentration in the aqueous phase. The water concentration in the aqueous phase,  $[H_2O]_{Aq}$ , was taken to be  $1 - [EtOH]_{Aq}$ . This assumption is valid for solvents with a low solubility in the aqueous phase, which are those of practical interest for use in solvent extraction processes. Additional details can be found in Offeman et al. [28]. A difference between the method used here and that presented previously is the

**Table 1**  
Solvents investigated

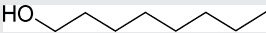
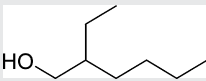
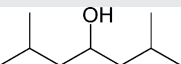






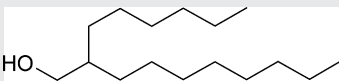

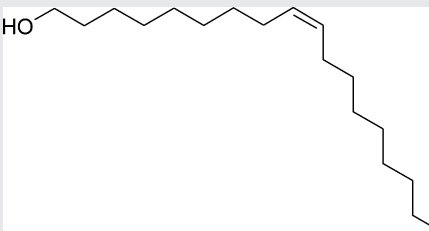
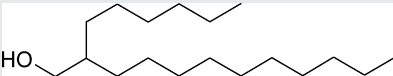
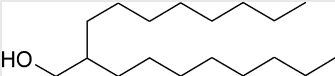
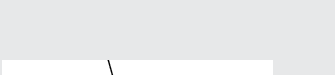
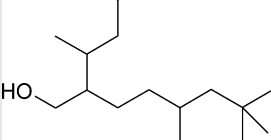
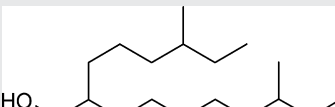


Name	Source	Composition	CAS	s.g., 20/20 °C	Structure
1-Octanol	Aldrich	99.93% 1-octanol	111-87-5	0.825	
Isooctyl alcohol	Eastman	99.84% 2-ethyl-1-hexanol	104-76-7	0.831	
2,6-Dimethyl-4-heptanol	Acros	Mixture: 90% 2,6-dimethyl-4-heptanol 10% 4,6-dimethyl-2-heptanol	108-82-7 51079-52-8	0.810	
1-Decanol	Fluka	99.3% 1-decanol	112-30-1	0.829	
1-Dodecanol	Aldrich	98.52% 1-dodecanol	112-53-8	0.830	
2-Butyl-1-octanol	Aldrich	97.9% 2-butyl-1-octanol	3913-02-8	0.832	
Isofol 14T	Sasol	Mixture:		0.834	
		23% 2-hexyl-1-octanol	19780-79-1		
		23% 2-butyl-1-decanol	21078-81-9		
		33.9% 2-hexyl-1-decanol	2425-77-6		
Jarcol I-16	Jarchem Industries	17.4% 2-butyl-1-octanol	3913-02-8		
		98.7% 2-hexyl-1-decanol	2425-77-6	0.835	
FO-1600, iso-palmityl alcohol	Nissan Chemical	98.1% 2-hexyl-1-decanol (described as iso-hexadecanol)	2425-77-6	0.840	
Jarcol 95BJ	Jarchem Industries	93% oleyl alcohol	143-28-2	0.835	

Table 1 (Continued)

Name	Source	Composition	CAS	s.g., 20/20 °C	Structure
Jarcol I-18E, <i>iso</i> -stearyl alcohol	Jarchem Industries	Mixture	70693-04-8	0.837	
		45% 2-hexyl-1-dodecanol	110225-00-8		
		45% 2-octyl-1-decanol	45235-48-1		
		5% 2-octyl-1-dodecanol 5% 2-hexyl-1-decanol	5333-42-6 2425-77-6		
FO-180, <i>iso</i> -stearyl alcohol	Nissan Chemical	99.8% <i>iso</i> -octadecanol	36400-98-3	0.843	
FO-180N, <i>iso</i> -stearyl alcohol	Nissan Chemical	99.1% <i>iso</i> -octadecanol (less branched than FO 180)	27458-93-1 (generic <i>iso</i> -octadecanol)	0.847	
Jarcol I-20	Jarchem Industries	98.0% 2-octyl-1-dodecanol	5333-42-6	0.838	

**Table 2**  
Measured ethanol partition coefficients and separation factors

Solvent	$K_{DE}$ (S.D.)	$\alpha$ (S.D.)	No.
1-Octanol <sup>a</sup>	0.716 (0.016)	12.0 (0.47)	4
2-Ethyl-1-hexanol <sup>a</sup>	0.688 (0.017)	19.5 (0.25)	4
2,6-Dimethyl-4-heptanol	0.514 (0.001)	32.4 (0.88)	2
1-Decanol <sup>a</sup>	0.541 (0.022)	12.2 (0.26)	3
1-Dodecanol <sup>a</sup>	0.441 (0.008)	12.3 (0.11)	4
2-Butyl-1-octanol <sup>a</sup>	0.362 (0.012)	25.7 (0.40)	4
Isofol 14T	0.293 (0.002)	27.1 (0.08)	2
Jarcol I-16	0.261 (0.009)	27.8 (1.04)	4
FO-1600	0.266 (0.007)	27.0 (0.88)	3
Oleyl alcohol	0.306 (0.005)	16.1 (0.14)	2
Jarcol I-18E	0.225 (0.004)	28.0 (1.80)	2
FO-180	0.253 (0.018)	34.9 (2.26)	8
FO-180N	0.243 (0.007)	32.5 (0.97)	4
Jarcol I-20	0.207 (0.001)	28.0 (1.38)	2

<sup>a</sup> Combined data of Offeman et al [18] and additional extractions performed subsequently for each solvent.

use of 1-pentanol or benzyl alcohol rather than 1-butanol as the organic phase diluent, and in the case of 2,4-dimethyl-4-heptanol, the use of 1-octanol instead of 1-hexanol as the internal standard.

## 2.2. Solvents and materials

Sources and characterization of the extraction solvents are shown in Table 1. These materials were used as received. Jarcol<sup>TM</sup>, Fine Oxocol<sup>TM</sup>, and Isofol<sup>TM</sup> are trademarks of Jarchem Industries, Inc., Nissan Chemical Industries, Ltd., and Sasol, Ltd., respectively. The CAS numbers shown for Jarcol I-18E, and Nissan Chemical's FO-180 and FO-180N do not correspond to unique structures, but rather to *iso*-alkanols of unspecified branching. The structures shown for these, however, were supplied by the manufacturers and indicate the true structures of the materials used. As can be seen from the structures in Table 1, Jarcol I-16 and Fine Oxocol FO-1600 appear to be the same compound, though the sources and purities differ.

Ethanol used in the extractions was from Aaper Alcohol and Chemical Co., >99.5%, undenatured (labeled 200 proof, absolute, anhydrous, ACS/USP grade). For the analysis, the organic phase diluent was 1-pentanol (Aldrich, 99.73%) or benzyl alcohol (Aldrich, 99.99%), which were stored over 3A molecular sieves to maintain dryness. The aqueous phase internal standard was 1-butanol (Aldrich, 99.95%), and the organic phase internal standard was 1-hexanol (Aldrich, 99.49%) or 1-octanol (Sigma, 98.9%). Distilled water was used in all solutions.

## 2.3. Yeast toxicity evaluation

The evaluation of the solvent toxicity or inhibition to yeast follows that of the shake flask biocompatibility tests described by Kollerup and Daugulis [29]. Briefly, shake flasks containing a sterilized glucose-based growth medium were inoculated with fermentation broth from a 24-h-old yeast culture and incubated at 30 °C in a rotary shaker bath for 8 h. At this point the cells were vigorously growing and 10 ml of solvent was added to the 55 ml culture in each flask. After a further 24 h, the flasks were sampled and analyzed for ethanol, residual glucose, dry cell weight, and cell viability. Results were compared to those of a solvent-free control culture. The yeast strain was Red Star<sup>®</sup> Ethanol Red<sup>TM</sup>, a strain of *Saccharomyces cerevisiae* that has been developed for the fuel alcohol industry, supplied by Fermentis, a division of S. I. Lesaffre Yeast Corp. It is described as a fast-acting, temperature tolerant dry yeast that displays higher alcohol yields and maintains higher cell viability during fermentation as compared with standard distiller's yeast.

## 3. Results and discussion

### 3.1. Ethanol and water partitioning performance

Ethanol extraction performance comparisons of solvents at fixed operating conditions can be conveniently represented by two parameters: ethanol distribution coefficient  $K_{DE}$  and separation factor  $\alpha$ . The ethanol distribution coefficient indexes the solvent's capacity for ethanol, while the separation factor is the solvent's selectivity for ethanol over water. The equilibrium distribution coefficient for ethanol is defined as the ratio of the weight percent of ethanol in the organic phase to the weight percent of ethanol in the aqueous phase:

$$K_{DE} = \frac{[EtOH]_{org}}{[EtOH]_{aq}} \quad (1)$$

The equilibrium distribution coefficient for water is defined similarly:

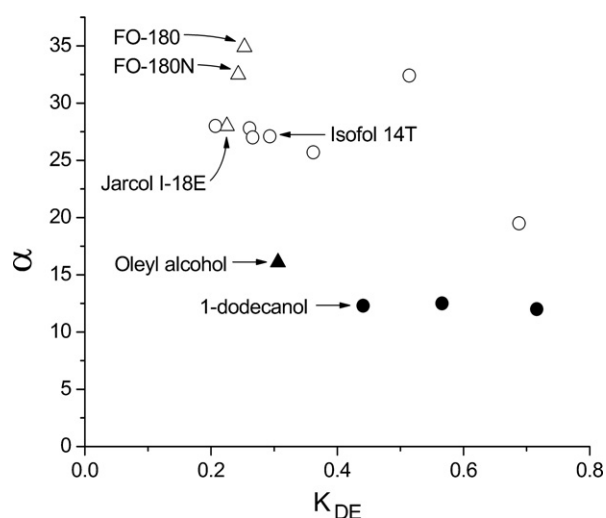
$$K_{DW} = \frac{[H_2O]_{org}}{[H_2O]_{aq}} \quad (2)$$

The separation factor is the ratio of ethanol to water in the organic phase divided by the ratio in the aqueous phase, or:

$$\alpha = \frac{K_{DE}}{K_{DW}} \quad (3)$$

The equilibrium ethanol capacity,  $K_{DE}$ , and the calculated separation factor,  $\alpha$ , for the solvents studied are shown in Table 2. The data for the C12 and smaller alcohols include data from our earlier works [18,28] combined with new extraction data on each solvent to reduce experimental variability. Standard deviations for the data are shown in the table. Comparisons with the literature are reasonable for the smaller alcohols, as discussed in Offeman et al. [28]. For the larger alcohols, oleyl alcohol was the only alcohol in this set found for literature comparison. Values of  $K_{DE}$  were reported as 0.21 [30], 0.24 [23], and 0.34 [31], and a single paper [30] reported  $\alpha$  as 22.8. These are compared to the  $K_{DE}$  and  $\alpha$  values determined in this work of 0.306 and 16.1, respectively. Differences between these reported values is not surprising, given the differences in purity of oleyl alcohol (93% used here, 74% and reagent grade used by others), the differences in operating conditions (aqueous phase ethanol concentration of 4–9 wt%, and extraction temperature of 20–65 °C), the presence or absence of fermentation broth components, and differences in analytical methods (GC, and enzyme assay).

Fig. 1 displays the  $\alpha$  and  $K_{DE}$  experimental data. As expected from previous observations about the effect of molecular weight,  $K_{DE}$  values for the C14–C20 alcohols are lower than those of the



**Fig. 1.** Ethanol extractive performance of aliphatic alcohol solvents at 33 °C and 5 wt% initial ethanol concentration. Filled symbols are unbranched 1-alcohols, open symbols are branched alcohols, triangles are C18 alcohols.

lower molecular weight alcohols.  $K_{DE}$  falls within a relatively narrow range for the C14–C20 alcohols, but separation factor is substantially increased by location of the hydroxyl group towards the middle of the molecule, and by branching of the alkyl chains. Oleyl alcohol, a C18 primary alcohol, has a low separation factor of 16.1; *iso*-octadecanol FO-180, the most highly branched of the C18 alcohols and with the hydroxyl group near the middle of the molecule, has a separation factor of 34.9, more than twice as large. The enhancement of  $\alpha$  by increased branching is displayed in Fig. 1 for the C18 alcohols (triangle symbol) in the progression from oleyl (unbranched) to I-18E (two terminal methyl groups) to FO-180N (four terminal methyls) to FO-180 (eight terminal methyls). These results show that the qualitative predictive guidelines [18] are effective in extrapolating to higher molecular weight alcohols.

### 3.2. Solvent retention in the aqueous phase

In a commercial solvent extraction process the phases are separated after the last extraction stage and the solvent phase is processed to recover the product and regenerate the solvent for reuse. The solubility of the solvent in the raffinate is an important consideration, and should be low, as it can represent a potential loss of solvent from the process. Make-up solvent must be purchased, and the lost solvent must be taken into account in the downstream processes, including waste treatment.

No binary data were found in the literature for the solubility in water of the  $\beta$ -branched C14–C20 alcohols studied here. Barton [32] shows a linear relationship between the logarithm of alcohol solubility and the carbon number for normal aliphatic alcohols at 298 K. By extrapolation, the solubility of 1-dodecanol in water is 4 ppm (mass). An update of the IUPAC-NIST data [33] shows solubility in the range of 2–3 ppm (mass). It is likely that the solubilities of the C14 and higher  $\beta$ -branched alcohols in water will be below 1 ppm (mass) at room temperature.

In our extraction studies, the C12–C20 alcohol solvents were not detectable by GC of the aqueous phase (typically containing ~4.5 wt% residual ethanol) after emulsification and phase separation. The detection limit was found to be 22 ppm for dodecanol, and 100 ppm for FO-180. A sample was made up containing 12% ethanol and equilibrated with dodecanol. The aqueous phase from this sample contained 40–60 ppm of dodecanol. The presence of 12% ethanol therefore raised the solubility of dodecanol in the aqueous phase by an order of magnitude. Ideally, solubility data for the solvent in fermentation broth would be preferred, at various ethanol concentrations, recognizing that if a multi-stage countercurrent contactor were used, the ethanol concentration in the raffinate could be quite low. Solvent solubility in water can be indicative, but solvent solubility will be enhanced by the presence of residual ethanol, and perhaps by other organic media components, and decreased by most salts.

Stable associations (clusters) of solvent molecules may be present in the aqueous phase (*i.e.* micelles or pre-micellar associations). This behavior is expected to be more important for the larger alcohols, which may exhibit surfactant-like behavior. Solvent losses to the aqueous phase due to this effect are likely to be larger than those from simple solubility of the alcohol in water, but are very dependent on (1) generation of solvent clusters due to hydrodynamic forces at the solvent/aqueous phase interface that entrain solvent molecules or clusters into the aqueous phase and (2) removal of solvent clusters via coalescence and density difference. Both are dependent on the equipment and residence times used for the extraction and subsequent phase separation.

### 3.3. Solvent toxicity to yeast

Whether continuous, batch, fed-batch or sequenced-batch fermentation is employed, contact between the solvent and the yeast is virtually certain in extractive fermentation. Even in the case where the raffinate is not recycled back to the fermentor, the raffinate may be further processed and a portion recycled upstream of the fermentor to maintain a favorable water balance in the plant.

**Table 3**  
Solvent toxicity to yeast

Solvent	Number of carbons	Name	Ratios to controls			
			Ethanol produced	Glucose consumed	Cell dry weight	Cell viability, staining
	8	1-Octanol	0.07	0.34	0.97	0.00
	8	2-Ethyl-1-hexanol	0.06	0.05	0.50	0.00
	9	2,6-Dimethyl-4-heptanol	0.09	0.17	0.61	0.00
	10	1-Decanol	0.04	0.04	0.70	0.00
	12	1-Dodecanol	0.07	0.07	0.59	0.01
	12	2-Butyl-1-octanol	0.39	0.56	0.74	0.60
	14	Isofol 14T mixture	0.98	1.00	0.94	0.93
	16	2-Hexyl-1-decanol	0.98	1.00	1.11	0.93
	18	Oleyl alcohol	1.03	1.00	1.04	1.30
	18	<i>iso</i> -Stearyl alcohol (FO-180N)	0.99	1.00	1.01	0.95
	18	<i>iso</i> -Stearyl alcohol (FO-180)	1.01	1.01	0.74	1.00
	18	Jarcol I-18E mixture	0.96	0.76	1.10	0.55
	20	2-Octyl-1-dodecanol	0.99	1.01	1.39	0.99

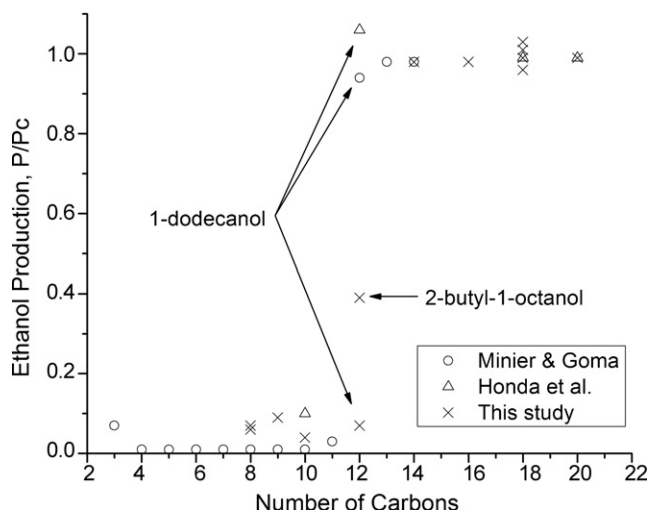


Fig. 2. Ratio of ethanol production to control for *S. cerevisiae* exposed to alcohol solvents containing varying numbers of carbons. Data from Minier and Goma [21], Honda et al. [23], and this study.

The results from the shaker flask experiments to determine each solvent's toxicity to yeast, or its inhibitory effects on cell growth or ethanol production, are shown in Table 3. Several lower molecular weight alcohols were included for comparison with the C14–C20 alcohols. There is a very clear difference between the results for the C8–C12 alcohols and the C14–C20 alcohols. Yeast contact with the lower molecular weight alcohols resulted in very low values of ethanol production and glucose consumption, cell weight, and cell viability by both staining and by plate count compared to the solvent-free control flasks. In fact, for all these solvents except for 2-butyl-1-octanol, the solvents were toxic to the cells. For the higher molecular weight alcohols, ethanol production, glucose consumption, cell growth, and cell viability were equivalent to the solvent-free controls.

The data for ethanol production, glucose consumption, and cell viability by the staining technique show low % errors of <10% between the nine solvent-free controls and for duplicated tests with solvents above C12. For C12 and below, % error is high because the average value is near zero. Cell dry weight and cell viability by plate count show much higher % errors in all cases and are less trustworthy.

Fig. 2 shows ethanol production (expressed as the ratio to the solvent-free control fermentation) for C3 through C20 alcohols. Data from two literature sources [21,23] compare favorably to data from this study. The only inconsistency is 1-dodecanol. It is important to note that the toxicity test used here is relatively harsh. There is a large solvent-to-aqueous phase ratio, the aqueous phase is saturated with solvent, and cells are exposed to the solvent/aqueous interface (often forming a gelled layer). Testing differences may explain the variability in the literature for alcohols in the transition region around C12, and in particular, the high toxicity observed in this test for 1-dodecanol relative to the other two studies referenced. For example, Kapucu and Mehmetoglu [34] were able to use the normally toxic *n*-decanol as an extraction solvent by encapsulating the yeast in calcium alginate beads along with 20% sunflower oil, thus preventing direct exposure of the cells to the water/solvent interface and reducing the solubility of the decanol in the beads via the encapsulated oil.

## 4. Conclusions

The C14–C20  $\beta$ -branched alcohols have a characteristic narrow range of  $K_{DE}$  values, but a large range of  $\alpha$  values, reflecting the influence of hydroxyl position and branching, as expected from performance guidelines developed previously. One example of this class, the C18 Fine Oxocol FO-180, was discovered in this work to have the highest value of  $\alpha$ , 34.9, of the alcohols studied. Although it would be very desirable to be able to use alcohol solvents with higher  $K_{DE}$  values than the 0.2–0.3 range typical of the C14–C20 alcohols, toxicity to fermenting yeast exposed to the solvents would preclude their use in extractive fermentations. On the other hand, the C14–C20 alcohol solvents have reduced solubilities in the raffinate compared to the smaller alcohols, and are non-toxic and non-inhibitory to the Ethanol Red™ yeast strain often used for fuel ethanol production. These results help to define and select viable performance regions for alcohols suitable for the recovery by solvent extraction of ethanol from aqueous solutions, and from yeast fermentation fluids in particular.

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